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14. ABSTRACT

Genomic profiling of the MCF7 breast cancer cell line using BRD4 ChIP-seq and DNase-seq revealed BRD4 ChIP-seq and DNase-seq to be highly similar. We discovered that the BET inhibitor JQ1 tends to be more effective in slowing the growth of basal rather than luminal breast cancer cell lines. The initial gene expression response of a basal breast cancer cell line, SUM159, on treatment with JQ1, is predominated by the down-regulation of gene expression and this down-regulation is highly associated with BRD4 occupied genomic loci. By 24h of JQ1 treatment secondary effects dominate the gene expression pattern and BRD4 binding no longer predicts gene expression changes. Long-term treatment of SUM159 with JQ1 results in this cell line becoming insensitive to JQ1 and adopting a gene expression pattern that is more luminal-like. This long-term change in gene expression is not highly associated with BRD4 binding.

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Therapeutic Mechanism of BET Bromodomain Inhibitor in Breast Cancer

Introduction

Epigenetic drugs that interact with chromatin regulators to modify the chromatin states of cancer cells promise to be important in cancer treatment (Yoo and Jones, 2006; Baylin and Jones, 2011). To effectively use these drugs to manipulate transcription for therapeutic purposes, we need to gain insight into their genome-wide function. We propose to develop both the experimental and computational methods and apply them to identify the efficacy, mechanism, and targets of BET domain inhibitor JQ1 in different breast cancer cells. This could potentially inform personalized breast cancer treatment based on tumor subtypes. The proposed experimental and computational approaches will be widely applicable to the development of other epigenetic drugs being developed to treat breast cancers.

Aim 1: Identify the cistrome, transcriptome and chromatin dynamics in a breast cancer cell line SUM159 treated with BET domain inhibitor JQ1.
Aim 2: Computationally infer and experimentally validate the JQ1 induced transcriptional regulatory network and pathway in SUM159.
Aim 3: Apply the above approaches to test JQ1 on different breast cancer cell lines and xenografts to compare the efficacy and specificity of JQ1 in different breast cancer cells.

Keywords

Breast cancer, bromodomain and extra terminal domain inhibitors, JQ1, luminal breast cancer, basal breast cancer, BRD4, Chip-seq

Overall Project Summary

Task 1. Identify cistrome, transcriptome and chromatin dynamics in the breast cancer cell line SUM159 treated with BET domain inhibitor JQ1 or BRD4 knockdown.

To optimize the DNase-seq protocol, we digested 2.5×10^6 breast cancer MCF-7 cells with 50 units of DNase I (Roche), and then selected DNA fragments in 50-100, 100-200, and 200-300bp sizes from each of the digested samples and barcoded to multiplex HiSeq sequencing. We carried out an analysis comparing the ability of data sets from different size fractions to discover transcription factor binding sites that had been identified by ChIP-seq. We found that for the estrogen receptor and CTCF short fragments were optimal (**Fig. 1**). Our analysis of the location of DNaseI cleavage sites with respect to nucleosome positions revealed that chromatin structure is likely to be the reason for the effectiveness of the short fragment DNase-seq.

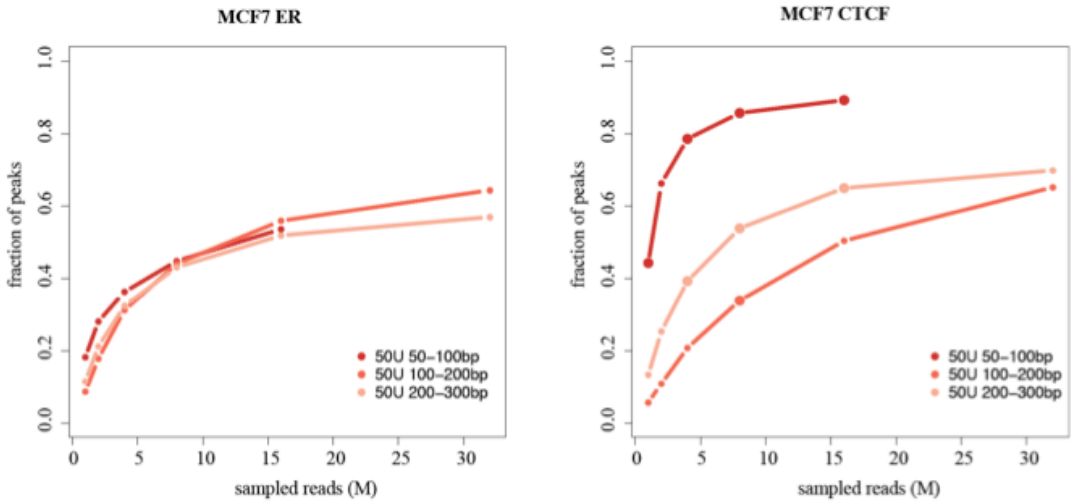

Figure 1. Fraction of estrogen receptor (ER) and CTCF ChIP-seq peaks in MCF7 identified as DNase-seq peaks for different fragment size selections. Short fragments are much more efficient than longer ones in recovering CTCF binding sites but this effect is less pronounced for the estrogen receptor.

Preliminary data (**Fig 2**) shows that DNase-seq data looks highly similar to BRD4 ChIP-seq and did not provide much new information about the influence of JQ1 on chromatin and transcriptional changes. This is a consistent with the known role of BRD4 in opening chromatin (Voigt and Reinberg, 2011), although the degree of similarity between BRD4 ChIP-seq and DNase-seq is remarkable. In subsequent analyses we will use H3K27ac, a mark that is indicative of enhancer activity, in addition to BRD4 ChIP-seq.

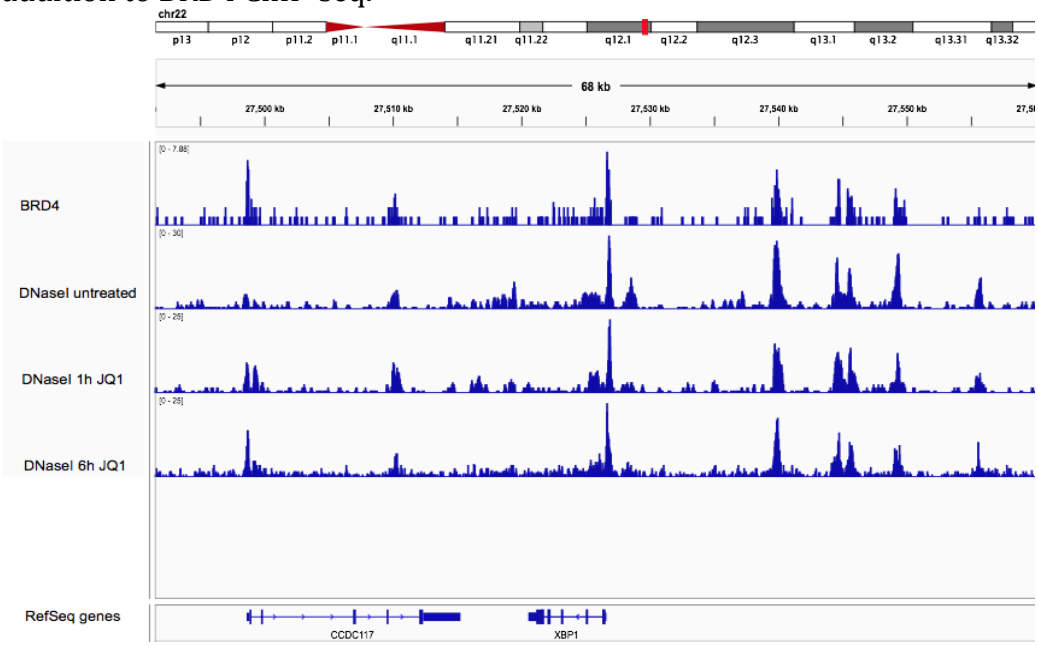

Figure 2. BRD4 ChIP-seq and DNase-seq in untreated MCF-7 cells and after 1h and 6h of JQ1 treatments. BRD4 ChIP-seq signal is very similar to DNaseI hypersensitivity and does not provide much complementary information.

Task 2: Computationally infer and experimentally validate the JQ1 induced transcriptional regulatory network and pathway in SUM159.

We conducted growth rate analyses on a panel of breast cancer cell lines treated by different concentrations of JQ1. This analysis revealed that basal breast cancer cell lines tend to be more responsive to JQ1 treatment than luminal cell lines. For this reason we decided to further study the treatment of the basal SUM159 cell line instead of the luminal MCF-7 cell line.

We analyzed the genome-gene expression profile of SUM159 after JQ1 treatment at 3h, 12h and 24h in relation to a control group treated with DMSO. This analysis revealed that genes were predominantly down-regulated after short term (3h) treatment by JQ1, as expected from current models of JQ1 action (Filippakopoulos et al., 2010). We used a model of "regulatory potential" to use BRD4 to predict the genes likely to be regulated by BRD4 and effected by JQ1 treatment. In this model a score is calculated for each gene as a sum of weights in which BRD4 ChIP-seq peaks close to the transcription start site of the gene are given more weight than BRD4 peaks that are far from the TSS of that gene. We evaluated the performance of this model in predicting gene expression changes associated with 3h,12h and 24h of JQ1 treatment and found that BRD4 ChIP-seq could inform which genes were down-regulated at 3h but could not predict the up-regulated genes. The performance of this model deteriorated with treatment time as secondary effects dominated the primary effect of BET inhibition. A similar analysis based on H3K27ac ChIP-seq data resulted in a poorer prediction for down-regulated genes but also some predictive power for up-regulated genes.

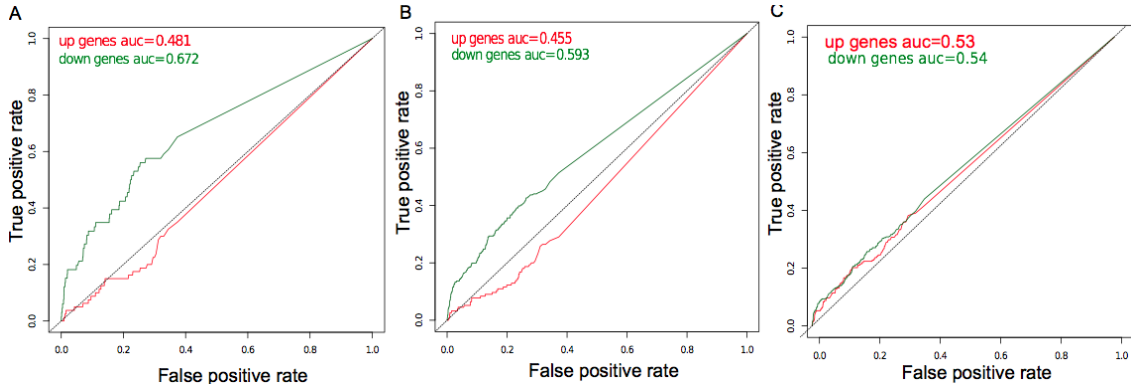

Figure 3. Regulatory potential model performance using BRD4 ChIP-seq to predict differentially expressed genes for 3h (A), 12h (B), and 24h (C) of JQ1 treatment. BRD4 ChIP-seq can predict early (3h) down-regulated genes but not up genes or late (24h) genes.

Task 3: Apply the above approaches to test JQ1 on different breast cancer cell lines and xenografts to compare the efficacy and specificity of JQ1 in different breast cancer cells.

We have collaborated with Dr Polyak's lab to assess the efficacy of JQ1 on different breast cancer cells lines and discovered that JQ1 tends to slow the growth of basal cell types more than luminal ones. In this collaboration we have also assessed the effect of long-term treatment of SUM159 cells with JQ1. After several months of treatment we find these JQ1 sensitive cells become less sensitive to JQ1. RNA-seq analysis of this JQ1 insensitive cell line reveals that the gene expression pattern appears to be more luminal than the parental SUM159 cells.

Key Research Accomplishments

- Optimized DNase-seq conditions and developed a model that explains fragment size effects in DNase-seq data.
- Analyzed and integrated BRD4 ChIP-chip and expression data in SUM159 cell line, before and after JQ1 treatment.
- Conducted H3K27ac histone mark ChIP-seq experiments in SUM159 cell lines.
- Established a JQ1 insensitive cell line from a parental cell line that is JQ1 sensitive.

Conclusion

To understand the mechanism of JQ1 resistance and its relationship with cell type we will conduct functional genomics and further epigenetic analyses. Specifically, we will perform a genome-wise CRISPR-Cas9 screen of genes in parental and resistant SUM159 cell lines, assessing which genes support or hinder cell proliferation in the presence and absence of JQ1. We will compare gene expression changes as well as BRD4 and H3K27ac ChIP-seq changes between parental and resistant cell lines with the genes that are revealed by this screen. Based on the results of the screen we will assess the effect of gene knock-outs on genome-wide gene expression to examine the extent to which these knock-outs can modulated the basal to luminal transition and thereby yield insight into the higher sensitivity of basal breast cancer cell types to the BET inhibitor JQ1.

Publications, Abstracts and Publications

None.

Inventions, Patents and Licenses

None.

Reportable Outcomes

None.

Other Achievements

None

References

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Appendices
Not applicable.